A new, flaccid, decurrent leaf variety of *Juniperus poblana* from Mexico: *J. poblana* var. *decurrens* R. P. Adams

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ABSTRACT

Analyses of nrDNA and four cp DNAs (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF) plus morphology and leaf essential oils revealed that the weeping (flaccid), decurrent leafed junipers near Topia, Durango are closely related to *J. poblana* (formerly *J. flaccida* var. *poblana*) and should be recognized as a new variety, *J. poblana* var. *decurrens* R. P. Adams **var. nov.** The leaf oil of *J. p.* var. *decurrens* is dominated by α-pinene (53.2%) with moderate amounts of β-pinene (5.3%), myrcene (4.3%), δ-2-carene (1.2%), δ-3-carene (2.5%), limonene (3.2%), β-phellandrene (3.1%), terpinolene (1.0%), (E)-caryphyllene (1.1%), and germacrene D (1.5%) and shares eleven unique terpenes with *J. poblana*. Published on-line www.phytologia.org *Phytologia 97(3): 152-163 (July 1, 2015).*

KEY WORDS: *Juniperus flaccida, J. martinezii, J. poblana, J. poblana* var. *decurrens* **var. nov.,** *Cupressaceae*, terpenes, leaf essential oil, morphology.

The flaccid leafed *Juniperus* of Mexico consist of three species: *J. flaccida* Schlecht. with large (9-12 mm diam.), multi-seeded [(4-)-6-10-(13)] cones; *J. poblana* (Martínez) R. P. Adams (formerly *J. flaccida* var. *poblana* Martínez) with very large (9-15 mm diam.), multi-seeded [(4-)-6-10-(13)] cones and *J. martinezii* Pérez de la Rosa with small seed cones (5-7 mm), 1-2 seeds per cone and foliage somewhat drooping but branchlets tips erect (Adams, 2014, Pérez de la Rosa. 1985). *Juniperus martinezii* is quite distinct in its morphology, but the other two taxa differ little in morphology with *J. flaccida* having radial branching and seed cones tan to brownish purple, whereas *J. poblana* has distichous foliage in vertical planes like *Thuja*, and not very flaccid (Zanoni and Adams, 1976, 1979; Adams, 2014) with bluish-brown seed cones. Each of these taxa has leaf margins that are hyaline and nearly entire, with either a few small teeth or merely a wavy margin (Adams, 2014). However, their DNA clearly places them in the serrate leaf margined *Juniperus* species of the western hemisphere with teethed margins secondarily lost (Adams, 2014).

Juniperus flaccida, J. martinezii and *J. poblana* have been treated as varieties of *J. flaccida*, until DNA sequencing of nrDNA (ITS) and trnC-trnD (Adams et al., 2006) revealed that *J. flaccida* varieties are not monophyletic and they recognized *J. f.* var. *martinezii* as *J. martinezii* and *J. f.* var. *poblana* as *J. poblana*. More recently, Adams and Schwarzbach (2013) published a detailed phylogeny of the serrate junipers of the western hemisphere based on nrDNA and four cp genes. They found *J. flaccida* (var. *flaccida*) in a group with *J. standleyi* (Fig. 1) and *J. poblana* (*J. f.* var. *poblana*) in a well supported sister group relationship. Likewise, *Juniperus martinezii* (*J. f.* var. *martinezii)* grouped with *J. durangensis* (Fig. 1) supported by high branch support. Their work appears to solidify support for the recognition of *J. martinezii* and *J. poblana.*

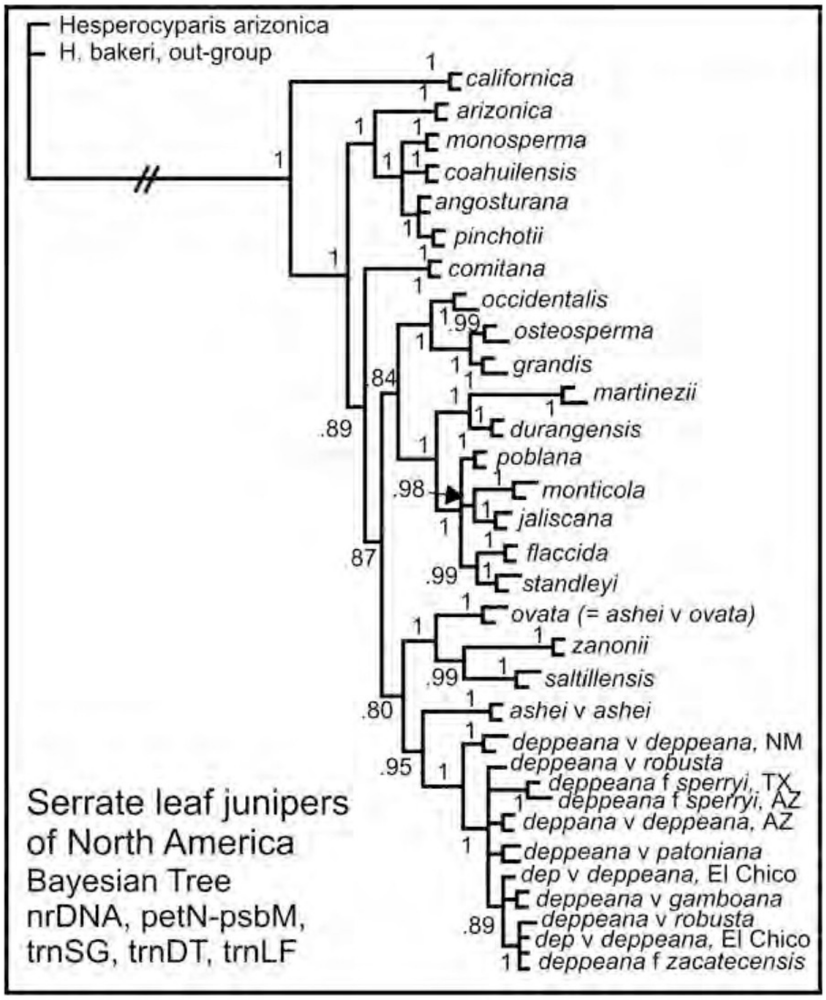

Figure 1. Bayesian analysis of the serrate leaf Juniperus of North America.
Numbers are posterior probabilities. From Adams and Schwarzbach (2013). See text for discussion.

The differences in morphology and oil composition warrant the recognition of the decurrent leafed, flaccid foliaged, *Juniperus* as a new variety:

Juniperus poblana var. ***decurrens*** R. P Adams , **var. nov.** TYPE: Mexico, Durango, 2 km s of Valle de Topia, 25° 14' 11" N; 106° 26' 55.7" W, 1818 m, *R. P. Adams 11926*, 30 Jun 2009 (HOLOTYPE: BAYLU), Fig. 2.

Similar to *Juniperus poblana* and *J. flaccida*, but differing in having only decurrent leaves with free, divergent leaf tips.

Juniperus poblana var. *decurrens* is currently known only from the type locality where it is common on hillsides around Topia at about 1550-2000 m.

Other specimens studied: TOPOTYPES: *Adams 11927, 11928* (BAYLU); *S. González, M. González, I. L. López e Ing. José Soto 7269a, b* (BAYLU, CIIDIR, MEXU); Los Pinos, Valle de Topia, *A. García 1336* (CIIDIR, MEXU).

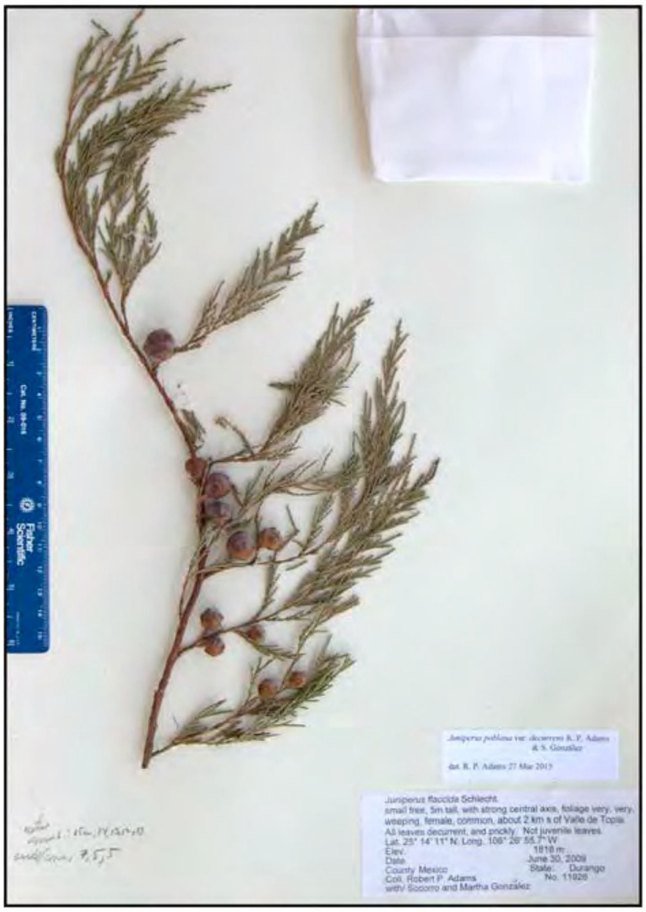

Fig. 2. Holotype of *J. poblana* var. *decurrens*.

Fig. 3. Leaves and seed cones of *J. poblana* var. *decurrens*.

Fig. 4. Habit of *J. poblana* var. *decurrens*.

Fig. 5. Leaf foliage, weeping.

Figure 6. Bark exfoliating in thin, scaly plates on *J. poblana* var. *decurrens*.

General description:
Dioecious. **Trees** to 10 m, branched with round crowns. **Trunk bark** brown exfoliating in thin, scaly plates. **Branches** very flaccid branchlets. **Leaves** all decurrent, with sharp, mucronate, usually divergent tips. **Seed cones** spherical, glaucous, bluish brown, 12-17 mm, mature cones usually show suture lines from fusion of cone-scales, appearing like a soccer ball. **Seeds** (4-)5-7(-9) per cone. **Pollen shed** spring. **Habitat** usually on dry slopes, in pure stands or in mixed forests, at 1550-2000 m elevation. **Uses** none known. **Dist**.: known only from type locality, Topia, Durango, Mexico. **Status**: limited distribution in areas that may be cleared for ranching, so it may become threatened in the future.

The purpose of the present paper is to compare the DNA sequences, volatile oils and morphology of *J. flaccida, J. martinezii*, *J. poblana* var. *poblana*, and *J. p.* var. *decurrens*.

The composition of the volatile leaf oils of *J. flaccida* and *J. poblana* (as *J. f.* var. *poblana*) were first reported by Adams, Zanoni and Hogge (1984). The composition of the leaf oil of *J. martinezii* was reported by Adams, Pérez de la Rosa and Cházaro (1990). Recently, Adams and Zanoni (2015) have reported on a re-examination the leaf oils of *J. flaccida, J. martinezii and J. poblana* using modern TIC-GC quantitation methods.

MATERIALS AND METHODS

Specimens collected: *Juniperus poblana* var. *decurrens, R. P. Adams 11926, 11927, 11928,* small trees, to 5 m tall, with strong central axis, foliage very, very, weeping, common, about 2 km s of Valle de Topia. All leaves decurrent, and prickly. Not merely juvenile leaves. 25° 14' 11" N; 106° 26' 55.7" W, 1818 m, 30 Jun 2009, Durango, Mexico; *J. flaccida* var. *flaccida*, *Adams 6892-6896*, 23 km e of San Roberto Junction on Mex. 60, Nuevo Leon, Mexico; *J. martinezii*, *Adams 5950-5952, 8709*, 40 km n of Lagos de Moreno on Mex. 85 to Amarillo, thence 10 km e to La Quebrada Ranch, 21º 33.08' N, 101º 32.57' W, Jalisco, Mexico; *J. poblana*, *Zanoni 2637-2643*, 0.74 mi N of Amozoc on old Rt. 150, Puebla, MX; *Adams 6868-6870*, 62 km s of Oaxaca, Mexico on Mex. 190.
Voucher specimens are deposited at BAYLU.

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 ºC until analyzed. The extracted leaves were oven dried (100 ºC, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20^{o} C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

DNA Amplifications and purification: see Adams, Bartel and Price (2009) and Adams and Kauffmann (2010). Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters. Available from http://www.geneious.com/) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data, using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing nrDNA, petN-psbM, trnS-trnG, trnD-trnT, and trnL-trnF resulted in 4351 bp of data. Adding these data from *J. poblana* var. *decurrens* to other serrate leaf junipers of North America, gave 62 OTUs for Bayesian analysis. This analysis revealed that *J. p.* var. *decurrens* is in a clade with *J. poblana* (Fig. 7), and thence in a clade with a morphologically diverse group of junipers (*J. flaccida, J. standleyi, J. monticola* and *J. jaliscana*).

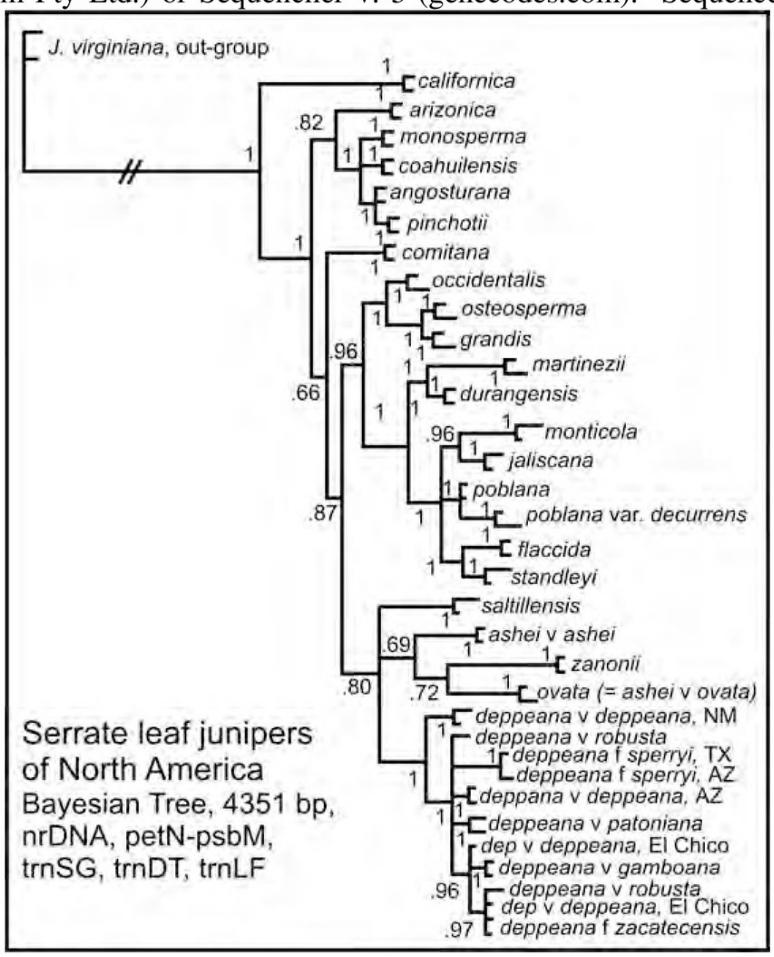

Fig. 7. Bayesian tree. Numbers next to the branch points are Posterior probabilities (0-1 scale).

To examine the magnitude of the DNA differences, a minimum spanning network was constructed based on differences in mutational events (MEs = SNPs + indels). Only 4 MEs separate *J. poblana* and *J. p.* var. *decurrens* (Fig. 8). These 4 MEs consist of 2 SNPs in the nrDNA, and 1 SNP and 1 indel (1bp) in cp DNA. Notice that *J. flaccida* and *J. p.* var. *decurrens* are separated by 10 MEs (4 SNPs + 1 indel in nrDNA and 1 SNP + 4 indels in cpDNA). *Juniperus poblana* occupies a central node among a number of morphologically diverse species (Fig. 8).

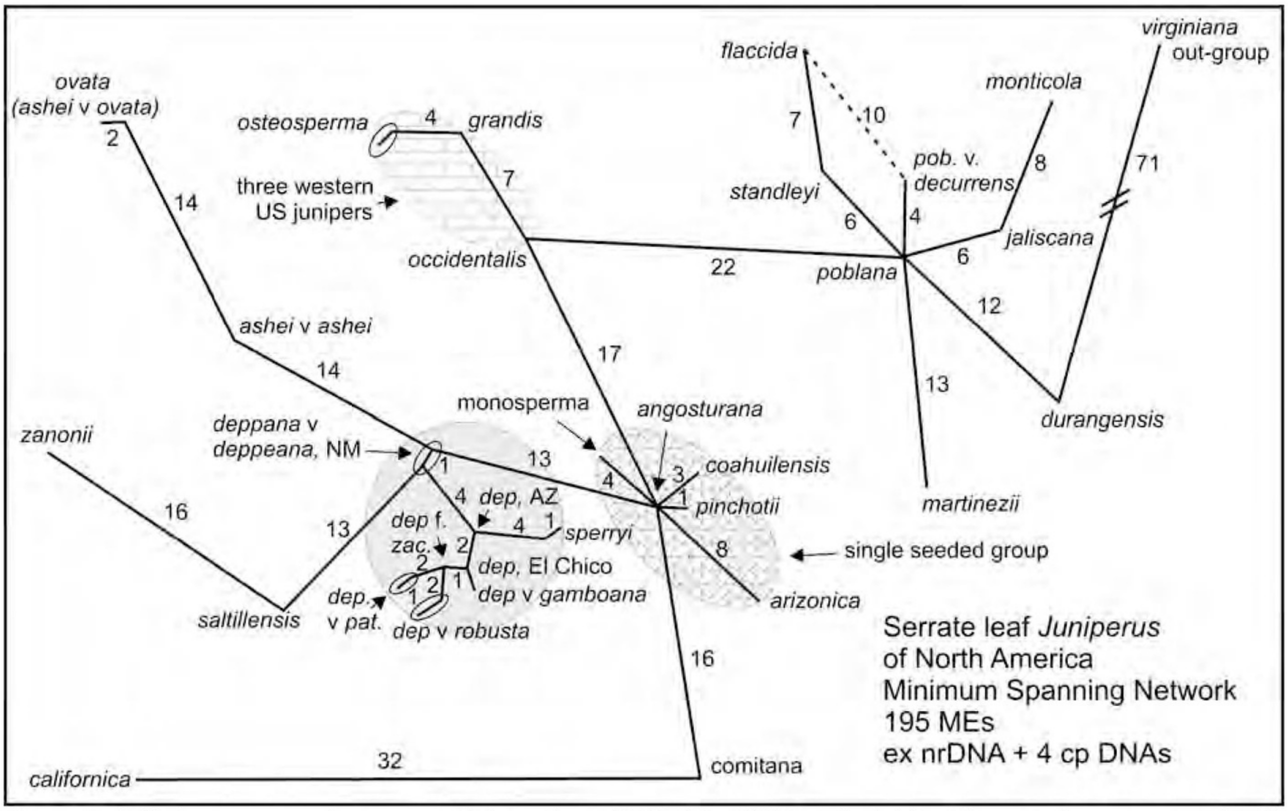

Figure 8. Minimum spanning network based on 195 MEs (SNPs + indels). The numbers next to the links are the number of MEs. The dashed line is the link between *J. flaccida* and *J. p.* var. *decurrens* (10 MEs) and is not a minimum link.

Analysis of the volatile leaf oils of *J. p.* var. *decurrens*, *J. poblana*, *J. flaccida* and *J. martinezii* is given in Table 1. Overall, the leaf oils of *J. flaccida* and *J. poblana* are similar and the oil of *J. martinezii* is quite different. The leaf oil of *J. p.* var. *decurrens* is dominated by α-pinene (53.2%) with moderate amounts of β-pinene (5.3%), myrcene (4.3%), δ-2-carene (1.2%), δ-3-carene (2.5%), limonene (3.2%), β-phellandrene (3.1%). terpinolene (1.0%), (E)-caryphyllene (1.1%), and germacrene D (1.5%). The leaf oil of *J. flaccida* is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.5%). The oil of *J. poblana* is somewhat similar as it is dominated by α-pinene (52.9%) with moderate amounts of β-pinene (4.2%), myrcene (4.3%), limonene (2.2%), β-phellandrene (3.5%) and linalool (1.6%). It contains only one unique compound: trans-verbenol (2.7%). The oil of *J. martinezii* was quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%), linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain several unique compounds: p-cymenene (0.7%), karahanaenone (1.3%), trans-dehydrocarvone (0.6%), trans-chrysanthenyl acetate (0.5%), linalool acetate (0.4%), noe0iso-3-thyjanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), trans-muurola-4(14), 5-diene (0.7%), epi-cubebol (0.5%), cubebol (1.1%), 1-epi-cubebol (1.0%), and an unknown diterpene (KI 1978, 0.6%).

It is interesting that *J. poblana* and *J. p.* var. *decurrens* have similar amounts of α-pinene (52.6, 53.2%) and share eleven unique components: δ-2-carene, δ-3-carene, endo-fenchol, methyl chavicol, elemicin, (E)-nerolidol, epi-α-eudesmol, epi-α-muurolol, α-cadinol, KI2264 (diterpene) and trans-ferruginol (Table 1).

The morphology of the leaves of *J. p.* var. *decurrens* is particular. There are three principal leaf types in *Juniperus* (Fig. 9): acicular (sections *Caryocedrus* and *Juniperus*); decurrent (section *Sabina*) and scale-like leaves (section *Sabina*). Within section *Sabina* there are several subtypes of leaves (Fig. 10).

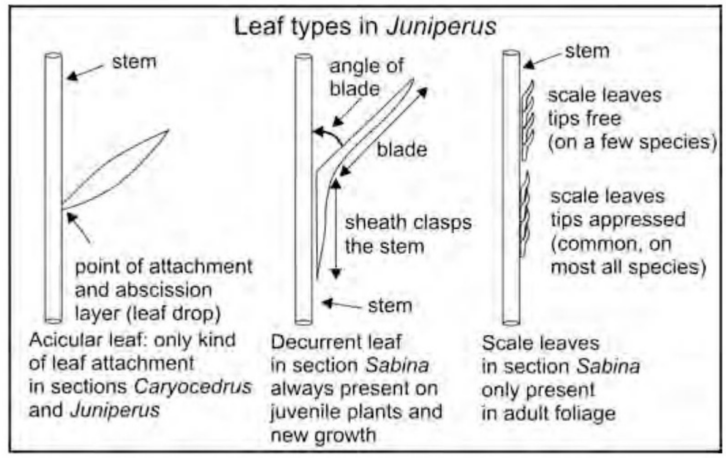

Figure 9. Three basic leaf types in *Juniperus*.

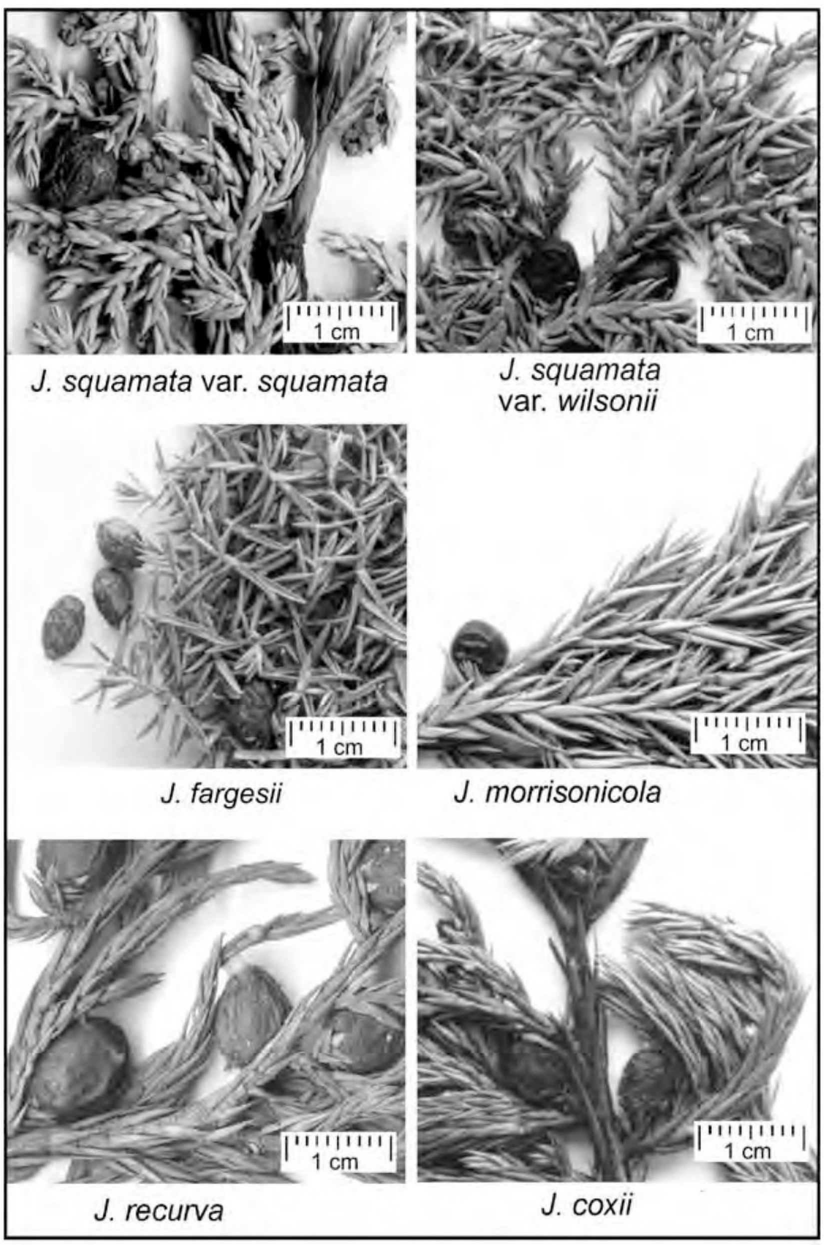

Figure 10. Variation in leaf types in section *Sabina* (left). Some of these types are confined to a species and some vary within and among species. None of these taxa have typical scale leaves (see *J. standleyi*, insert below). Note also decurrent leaves of *J. poblana* var. *decurrens* below.

Decurrent leaves of *J. poblana* var. *decurrens*

A comparison of the morphology of *J. poblana, J. p.* var. *decurrens* and *J. flaccida* is given in Table 2. The taxa are difficult to separate. A key to these taxa (plus *J. martinezii*) follows:

1. Seed cones large, 9-17 mm diam., (4-) 6-10 (-13) seeds per cone, terminal branch tips drooping (hanging)
    2. Foliage flaccid, but not weeping, branching planate, seed cones bluish-brown .... *J. poblana* var. *poblana*
    2. Foliage weeping, branching radially, seed cones brownish-purple, tan-brown
        3. All leaves decurrent, with free tips, foliage very weeping, bark exfoliating in thin, scaly, plates .... *J. poblana* var. *decurrens*
        3. Leaves decurrent with mostly appressed tips, foliage weeping, bark exfoliating in thick, inter-laced stripes.. *J. flaccida*
1. Seed cones small, (5-) 6 (-9) mm diam., 1-2 (-3) seeds per cone, terminal branch tips erect .... *J. martinezii*

Table 2. Comparison of morphology of *J. poblana, J. p.* var. *decurrens* and *J. flaccida.*

	J. poblana var. *poblana*	*J. p.* var. *decurrens*	*J. flaccida*
leaves	decurrent leaves (DL) with free tips and some modified DL with appressed tips.	decurrent leaves (DL) with free tips and many modified DL with free tips.	decurrent leaves (DL) with free tips and many modified DL with appressed tips.
leaf tips	mucronate tips on DL	mucronate tips on DL and mod. DL	mucronate tips on DL, acute on modified DL
leaf gland	about 1/2 DL length	about 1/2 DL length about 1/2 mod. DL length	about 3/4 DL length about 1/2 mod. DL length
bark exfoliation pattern	thin, narrow, interlaced strips	thin, scaly plates	thick, interlaced strips
seed cones	9-15 mm, bluish brown	12-17 mm, bluish brown to purplish brown	9-12 mm, tan-brown to brownish purple
seeds per cone	(4-) 6-10 (-13)	(4-) 5-7 (-9)	(4-) 6-10 (-13)

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Table 1. Leaf essential oil compositions for *J. flaccida* (*Adams 6892*), *J. poblana* var. *decurrens* (*Adams 11932*), *J. poblana* var. *poblana* (*Adams 2578*), and *J. martinezii* (*Adams 5974*) based on FID gas chromatography and GCMS identification. Those compounds that appear to distinguish taxa are in boldface.

KI	Compound	*flaccida*	*decurrens*	*poblana*	*martinezii*
921	**tricyclene**	**0.2**	**t**	**t**	**0.6**
924	**α-thujene**	**t**	**t**	**t**	**0.6**
932	**α-pinene**	**65.0**	**53.2**	**52.9**	**16.6**
945	α-fenchene	t	0.1	0.1	-
946	camphene	0.6	0.5	0.7	0.7
953	thuja-2,4-diene	t	t	0.2	0.1
961	verbenene	1.3	0.1	0.6	0.2
969	**sabinene**	**0.2**	**-**	**0.2**	**10.4**
974	1-octen-3-ol	-	0.1	-	-
974	**β-pinene**	**4.8**	**5.3**	**4.2**	**1.4**
988	myrcene	4.3	5.6	4.3	3.6
1001	**δ-2-carene**	**-**	**1.2**	**1.8**	**-**
1001	4-methyl, me-pentanoate*	0.1	-	-	-
1002	α-phellandrene	0.1	0.1	0.1	1.0
1008	**δ-3-carene**	**-**	**2.5**	**1.4**	**-**
1014	α-terpinene	t	t	t	1.0
1020	p-cymene	0.1	t	0.2	1.8
1024	limonene	3.5	3.2	2.2	1.8
1025	β-phellandrene	3.4	3.1	3.5	5.3
1032	(Z)-β-ocimene	t	0.1	t	t
1044	(E)-β-ocimene	1.5	1.8	0.7	0.4
1054	**γ-terpinene**	**0.2**	**0.1**	**0.1**	**1.8**
1065	cis-sabinene hydrate	-	-	-	0.6
1067	cis-linalool oxide (furanoid)	0.1	-	t	-
1086	terpinolene	0.5	1.0	0.7	0.8
1089	**p-cymenene**	**-**	**-**	**-**	**0.7**
1092	96, 109,43,152, C10-OH	1.0	-	0.3	1.8
1095	**linalool**	**2.9**	**0.7**	**1.6**	**2.8**
1112	3-m-3-buten-me-butanoate	0.2	-	-	-
1114	endo-fenchol	-	0.1	0.3	-
1118	cis-p-menth-2-en-1-ol	0.1	0.1	0.2	0.5
1122	α-campholenal	0.3	0.1	1.2	0.4
1133	cis-p-mentha-2,8-dien-1-ol	-	-	t	-
1135	trans-pinocarveol	0.3	-	1.1	0.8
1136	trans-p-menth-2-en-1-ol	-	0.2	-	-
1141	**camphor**	**0.5**	**0.3**	**0.6**	**11.1**
1141	**trans-verbenol**	**-**	**-**	**2.7**	**-**
1145	camphene hydrate	0.4	0.2	0.5	1.3
1148	citronellal	0.2	-	t	-
1154	**karahanaenone**	**-**	**-**	**-**	**1.3**
1155	iso-isopulegol	0.1	-	-	-
1160	**p-mentha-1,5-dien-8-ol**	**-**	**-**	**0.5**	**1.0**
1165	**borneol**	**0.7**	**0.7**	**0.6**	**-**
1172	cis-pinocamphone	0.2	0.1	0.3	0.3
1174	**terpinen-4-ol**	**0.3**	**0.2**	**0.3**	**6.1**
1178	**naphthalene**	**-**	**0.4**	**t**	**t**
1179	**p-cymen-8-ol**	**t**	**t**	**t**	**0.5**
1186	α-terpineol	0.4	0.9	0.7	0.7
1195	myrtenol	0.1	-	0.2	t
KI	Compound	flac6892	fjuv1932	pob2578	mart5974
1195	myrtenal	-	-	-	0.1
1195	**methyl chavicol**	**-**	**0.8**	**0.7**	**-**

KI	Compound	*flaccida*	*decurrens*	*poblana*	*martinezii*
1200	trans-dehydrocarvone	-	-	-	0.6
1204	**verbenone**	**t**	**t**	**0.6**	**0.5**
1215	**trans-carveol**	**0.1**	**-**	**0.7**	**-**
1218	endo-fenchyl acetate	-	0.1	-	-
1223	citronellol	0.1	-	-	-
1232	thymol, methyl ether	-	0.1	-	-
1235	**trans-chrysanthenyl acetate**	**-**	**-**	**-**	**0.5**
1239	carvone	-	-	0.2	-
1249	**piperitone**	**0.2**	**0.1**	**0.9**	**0.9**
1254	**linalool acetate**	**-**	**-**	**-**	**0.4**
1255	4Z-decenol	0.2	-	-	-
1284	bornyl acetate	0.4	0.8	1.1	1.8
1289	trans-sabinyl acetate	-	-	-	0.1
1289	**neo-iso-3-thyjanly acetate**	**-**	**-**	**-**	**0.8**
1289	thymol	-	-	0.2	-
1292	(2E,4Z)-decadienal	0.1	-	-	-
1315	(2E,4E)-decadienal	0.1	-	-	-
1320	**aromatic phenol 149,91,77,164**	**-**	**-**	**-**	**0.6**
1344	myrtenyl acetate	-	-	0.1	-
1345	α-terpinyl acetate	-	-	-	0.2
1345	α-cubebene	0.1	-	0.1	0.3
1396	duvalene acetate	-	0.3	-	-
1403	methyl eugenol	0.1	0.3	-	-
1417	**(E)-caryophyllene**	**0.2**	**1.1**	**0.3**	**0.1**
1448	cis-muurola-3,5-diene	-	0.2	-	-
1451	trans-muurola-3,5-diene	-	-	-	0.2
1452	α-humulene	-	-	t	-
1475	trans-cadina-1(6),4-diene	-	0.1	-	0.3
1484	**germacrene D**	**0.1**	**1.5**	**0.3**	**-**
1493	**trans-muurola-4(14),5-diene**	**-**	**0.1**	**-**	**0.7**
1493	**epi-cubebol**	**-**	**-**	**-**	**0.5**
1500	α-muurolene	-	-	t	-
1513	γ-cadinene	-	0.2	-	-
1514	**cubebol**	**-**	**0.4**	**-**	**1.1**
1521	trans-calamenene	-	-	t	0.5
1522	δ-cadinene	-	0.4	t	0.4
1528	zonarene	-	0.1	-	0.1
1533	trans-cadina-1,4-diene	-	-	-	t
1548	elemol	0.1	-	0.2	1.0
1555	elemicin	-	0.4	0.2	-
1561	**(E)-nerolidol**	**-**	**0.9**	**2.5**	**-**
1582	caryophyllene oxide	0.2	0.8	0.6	0.3
1627	**1-epi-cubenol**	**-**	**0.7**	**-**	**1.0**
1630	γ-eudesmol	-	-	-	t
1638	epi-α-cadinol	-	0.8	0.1	-
1638	epi-α-muurolol	-	0.8	0.1	-
1649	β-eudesmol	-	-	t	0.3
1652	α-eudesmol	-	-	0.1	0.3
1652	α-cadinol	-	0.8	0.1	-
1685	germacra-4(15),5,10-triene-1-al	-	0.8	-	-
1759	benzyl benzoate	-	t	-	-
1933	cyclohexadecanolide	-	t	-	-
1958	**iso-pimara-8(14),15-diene**	**0.1**	**-**	**-**	**1.0**
1978	**diterpene,43,81,147,243**	**-**	**-**	**-**	**0.6**
1987	manoyl oxide	3.0	0.6	0.3	1.0
KI	Compound	flac6892	fjuv1932	pob2578	mart5974
2055	abietatriene	0.3	0.1	0.2	0.8

KI	Compound	*flaccida*	*decurrens*	*poblana*	*martinezii*
2087	**abietadiene**	**-**	**-**	**-**	**2.3**
2056	manool	-	0.1	-	-
2105	iso-abienol	-	-	0.1	-
2264	**diterpene,43,55,271,286**	**-**	**0.8**	**t**	**-**
2331	trans-ferruginol	-	0.2	t	-

KI = Kovats Index (linear) on DB-5 column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.